[Chem. Pharm. Bull.]
[29(7)1870-1875(1981)]

Synthesis of 2-Phenylaminoadenosine from Imidazole Nucleosides

KIYOSHI OMURA, RYUJI MARUMOTO, and YOSHIYASU FURUKAWA*

*Central Research Laboratories, Takeda Chemical Industries, Ltd.,
Jusohonmachi, Yodogawa-ku, Osaka 532, Japan*

(Received January 16, 1981)

Three methods for the synthesis of 2-phenylaminoadenosine (1, CV-1808), a potent coronary vasodilator with prolonged action, were exploited.

1) The reaction of 5-amino-4-cyano-1-(β -D-ribofuranosyl)imidazole (7) with phenyl isothiocyanate gave 7-imino-5-phenylamino-3-(β -D-ribofuranosyl)imidazo[4,5-*d*][1,3]thiazine (11), which, on alkaline treatment, rearranged to 6-mercapto-2-phenylamino-9-(β -D-ribofuranosyl)purine (12). On methylation, 12 gave the 6-methylmercapto derivative (14), which was converted to 1 by treatment with ammonia.

2) 5-Amino-4-cyano-1-(β -D-ribofuranosyl)imidazole (7) reacted with phenyl cyanamide in methanolic ammonia, giving 1 and 2-aminoadenosine as a by-product.

3) Ethyl 5-amino-1-(β -D-ribofuranosyl)-4-carboximidate (21b) was directly obtained by treatment of 5-amino-1-(2,3,5-tri-O-propionyl- β -D-ribofuranosyl)imidazole-4-carboxamide (4) with Meerwein's reagent followed by deacylation, and this was led to 1 by the reaction with phenyl cyanamide.

Keywords—CV-1808; 2-phenylaminoadenosine; phenyl cyanamide; imidazole nucleoside; coronary vasodilator; imidazo[4,5-*d*][1,3]thiazine

Adenosine is well known to possess coronary vasodilatory action and has been proposed to be a physiological mediator for regulating coronary vascular tone.¹⁾ Because of its rapid uptake into erythrocytes and myocardial tissue, and its conversion to nucleotides by adenosine kinase or to inosine by adenosine deaminase,²⁾ its therapeutic application has been limited to the intraarterial route.

In order to obtain compounds having long-lasting action even on oral administration, many N⁶- and 2-substituted adenosines³⁾ and adenosine derivatives with a modified ribose moiety⁴⁾ have been synthesized, and some of them were shown to have stronger and longer-lasting activities than adenosine.

We have reported the synthesis and coronary vasodilatory action of 2-(substituted amino)-adenosines,⁵⁾ among which 2-phenylaminoadenosine (1, CV-1808) showed most desirable activity on oral administration.⁶⁾ Compound 1 has been synthesized from 5-amino-1-(β -D-ribofuranosyl)imidazole-4-carboxamide (2) which is available in quantity⁵⁾ as follows: 2→2-mercaptinosine→2-brominosine→2-phenylamininosine→2',3',5'-tri-O-acetyl-2-phenylamininosine→2',3',5'-tri-O-acetyl-6-chloro-2-phenylaminonebularine→1. However, this method consists of six steps and the overall yield is low. The present study deals with new, facile synthetic methods for 1.

In the first step⁷⁾ of the above synthesis, 5-(N'-phenylthioureido)-1-(β -D-ribofuranosyl)-imidazole-4-carboxamide (3) was assumed to be an intermediate, but it could not be isolated. In order to detect this intermediate, 5-amino-1-(2,3,5-tri-O-propionyl- β -D-ribofuranosyl)-imidazole-4-carboxamide (4) was allowed to react with phenyl isothiocyanate in dimethylformamide (DMF) to give the expected 5-(N'-phenylthiocarbamoyl)amino-1-(2,3,5-tri-O-propionyl- β -D-ribofuranosyl)imidazole-4-carboxamide (5), which, under heating, rapidly liberated aniline to give 2',3',5'-tri-O-propionyl-2-mercaptinosine (6) (Chart 1). In view of this finding it was expected that 1 could be synthesized by the reaction of 5-amino-4-cyano-1-(β -D-ribofuranosyl)imidazole⁸⁾ (7) with phenyl isothiocyanate followed by methylation and ammonolysis (8→9→10) (Chart 1).

Chart 1

The reaction of 7 with phenyl isothiocyanate in DMF gave yellow crystals, which were assumed to be 5-(N'-phenylthioureido)-4-cyano-1-(β -D-ribofuranosyl)imidazole (8) on the basis of their elemental analysis. However, inspection of the IR spectrum revealed no peak near 2200 cm^{-1} due to the cyano group. This nucleoside was identified as 7-imino-5-phenylamino-3-(β -D-ribofuranosyl)imidazo[4,5-d][1,3]thiazine (11) by conversion of it into 6-mercapto-2-phenylamino-9-(β -D-ribofuranosyl)purine (12). The structure of 12 was confirmed by comparison with an authentic sample synthesized by an unambiguous route, *i.e.*, thiolation of 2-phenylaminoinosine-triacetate (13) followed by deacetylation. We have previously reported a similar reaction of 7 with carbon disulfide in pyridine.⁵⁾ Similarly, certain cyclic *o*-amino-nitriles are known to give analogous ring closure compounds.⁹⁾ Compound 12 was methylated with methyl iodide to give 6-methylthio-2-phenylamino-9-(β -D-ribofuranosyl)purine (14),

Chart 2

which was heated in methanolic ammonia at 180°C for 20 h to give **1** (Chart 2). However, this method is not satisfactory, because the yield of the last step was low.

It is known that tautomerism of phenyl cyanamide between a cyanamide structure (A) and a carbodiimide structure (B) is possible.¹⁰⁾ The structure (B) seems to be a nitrogen isostere of phenyl isothiocyanate. Furthermore, addition reactions of phenyl cyanamide with nucleophiles are well known.¹¹⁾ Therefore, if **7** adds to phenyl cyanamide and the adduct (**10**) cyclizes to **1**, a one-step synthesis of **1** would be possible. In fact, in methanolic ammonia at 150°C, **1** was formed in about 40% yield [determined by thin-layer chromatography (TLC)] accompanied by an almost equal amount of 2-aminoadenosine¹²⁾ (**15**). Crystallization of the crude reaction product gave pure **1** in 21% yield. The yield of **1** was scarcely affected by the content of ammonia in the solvent. The same yield was obtained in methanolic propylamine, but in methanolic triethylamine complicated by-products were formed. Though detailed studies on the reaction mechanism are still lacking, **7** may either add to phenyl cyanamide or be displaced by phenylguanidine,¹³⁾ which may be formed from phenyl cyanamide and ammonia, to give 5-phenyldiaminomethyleneamino-4-cyano-1-(β -D-ribofuranosyl)imidazole (**10**), whose amino group attacks the cyano group to provide **1**. In the case of methanolic ammonia, displacement of the phenylamino group of **10** by ammonia may give 5-diaminomethyleneamino-4-cyano-1-(β -D-ribofuranosyl)imidazole (**16**), which cyclizes into **15** (Chart 3).

Chart 3

Similar reactions using N,N'-diphenyl carbodiimide (**17**) in place of phenyl cyanamide gave **1**, the possible intermediate being a guanidine derivative (**18**) (Chart 3). It is well known that phenyl cyanamide changes slowly into triphenylmelamine (**19**) and trianilino-s-triazine (**20**) under mild conditions.¹⁴⁾ Thus, the reactions of these compounds (**19**, **20**) with **7** were

examined and it was found that **1** was derived from **19**, and not from **20** (Chart 3). The reaction mechanism is not clear.

Although facile syntheses of **1** from **7** were achieved as described above, we sought yet another synthetic method for **1**. It seemed to us that methyl 5-amino-1-(β -D-ribofuranosyl)-imidazole-4-carboximate (**21a**) might react with phenyl cyanamide to give **1**.

Compound **21a** was first prepared by reacting **7** with methanolic hydrogen chloride.¹⁵⁾ We found that the reaction of **4** with Meerwein's reagent followed by deacylation with methanolic ammonia gave **21b** in good yield. Compound **21b** reacted with phenyl cyanamide in methanolic ammonia to give **1** in good yield (Chart 3).

In conclusion, we have found three new methods for the synthesis of **1**. The second method was most favorable, and about eighty analogs of **1** were synthesized by this method. Structure-activity relations of the analogs will be reported elsewhere.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-390 spectrometer (90 MHz) using tetramethylsilane as an external standard.

The following silica gels (E. Merck) were used: "DC Alufolien Kieselgel F₂₅₄" for thin-layer chromatography (TLC) and "Kieselgel 0.05—0.2 mm" for column chromatography.

5-Amino-1-(2,3,5-tri-O-propionyl- β -D-ribofuranosyl)imidazole-4-carboxamide (4)—A solution of **2** (258 g) in pyridine (350 ml) and propionic anhydride (400 ml) was stirred at room temperature for 16 h. The mixture was evaporated to dryness *in vacuo*, and the residue was triturated in H₂O (2.5 l) to give crystals, mp 115—116°C (355 g, 83%). Recrystallization of an aliquot from EtOH-Et₂O afforded colorless needles, mp 117—118°C. *Anal.* Calcd for C₁₈H₂₈N₄O₈: C, 50.70; H, 6.15; N, 13.14. Found: C, 50.60; H, 6.10; N, 13.21.

5-(N'-Phenylthioureido)-1-(2,3,5-tri-O-propionyl- β -D-ribofuranosyl)imidazole-4-carboxamide (5)—PhNCS (10 g) was added to a solution of **4** (5 g) in DMF (30 ml), and the solution was heated at 50°C for 5 h. The reaction mixture was diluted with H₂O (200 ml) then extracted with AcOEt (100 ml $\times$ 3), and the extract was washed with H₂O (100 ml $\times$ 3). The extract was evaporated to dryness and the residue was chromatographed on silica gel (100 g) using CHCl₃-MeOH (20:1) as an eluent. Fractions containing **5** were evaporated to dryness to give a colorless resin (2.17 g, 33%). NMR (CDCl₃) δ : 0.95—1.3 (9H, m, CH₃CH₂ $\times$ 3), 2.15—2.5 (6H, m, CH₃CH₂ $\times$ 3), 4.35 (3H, broad, H_{4'}, 2H_{5'}), 5.25 (1H, t, H_{3'}), 5.6—5.75 (1H, m, H_{2'}), 6.2 (1H, d, H_{1'}), 7.0—7.6 (5H, m, Ph), 7.7 (1H, s, H₂), 9.0 (1H, broad, NH), 9.6 (1H, broad, NH). *Anal.* Calcd for C₂₅H₃₁N₅O₈S: C, 53.47; H, 5.56; N, 12.47; S, 5.71. Found: C, 52.83; H, 5.47; N, 12.11; S, 5.68. The mass spectrum (MS) of this compound revealed a fragment peak (*m/e* 486, M⁺-PhNH₂) instead of the desired molecular ion peak (*m/e* 561). This indicates ready thermal conversion of **5** into **6**.

7-Imino-5-phenylamino-3-(β -D-ribofuranosyl)imidazo[4,5-*d*][1,3]thiazine (11)—PhNCS (25 g) was added to a solution of **7** (5 g) in DMF (50 ml) and the solution was set aside at room temperature for 4 days. After heating at 50°C for 8 h, the solution was cooled and diluted with H₂O (400 ml) and Et₂O (100 ml), whereupon a yellow crystalline solid separated. The solid was filtered off and thoroughly washed with H₂O and Et₂O. This product was dissolved in a minimum amount of DMF and the solution, on addition of Et₂O (200 ml), formed two layers. MeOH was added until a homogeneous solution was obtained. On standing at room temperature, crystals separated. They were collected and washed with EtOH and Et₂O to give yellow crystals (6.55 g, 88%), mp 150—170°C (dec.). NMR (DMSO-*d*₆) δ : 5.86 (1H, d, H_{1'}), 6.9—8.0 (5H, m, Ph), 8.20 (1H, s, H₈), 10.52 (1H, s, PhNH), 10.8—11.4 (1H, NH). *Anal.* Calcd for C₁₆H₁₇N₅O₄S: C, 51.20; H, 4.57; N, 18.66. Found: C, 51.50; H, 4.36; N, 18.06. MS *m/e*: 375 (M⁺).

6-Mercapto-2-phenylamino-9-(β -D-ribofuranosyl)purine (12)—A mixture of **11** (4 g) and 1 N NaOH (40 ml) was heated at 100°C for 30 min and after cooling, the solution was slightly acidified with 10% aq. HCl to deposit a pale yellow powder (3.13 g, 78%), which was recrystallized from 70% MeOH to give pale yellow crystals, mp 235—240°C (dec.). NMR (DMSO-*d*₆) δ : 5.75 (1H, d, H_{1'}), 6.9—7.7 (5H, m, Ph), 8.20 (1H, s, H₈), 9.0 (1H, s, PhNH), 11.9 (1H, s, NH or SH). *Anal.* Calcd for C₁₆H₁₇N₅O₄S: C, 51.20; H, 4.57; N, 18.66. Found: C, 51.17; H, 4.59; N, 18.67. MS *m/e*: 375 (M⁺).

6-Methylthio-2-phenylamino-9-(β -D-ribofuranosyl)purine (14)—MeI (160 mg) was added to a solution of **12** (375 mg) in 1 N NaOH (1.2 ml). The solution soon began to precipitate colorless crystals, which were filtered off and washed with H₂O sufficiently to give **14** (0.23 g, 83%), mp 245—248°C. *Anal.* Calcd for C₁₇H₁₉N₅O₄S: C, 52.44; H, 4.92; N, 17.99. Found: C, 52.33; H, 5.03; N, 17.50. NMR (DMSO-*d*₆) δ : 2.67 (3H, s, Me), 5.88 (1H, d, H_{1'}), 6.7—7.9 (5H, m, Ph), 8.28 (1H, s, H₈), 9.42 (1H, s, PhNH). Compound **14** (150 mg) in 20% NH₃-MeOH (10 ml) was heated at 180°C for 3 h in an autoclave. The yield of **1** was determined by TLC (solvent, CHCl₃-MeOH (2:1)) to be 30%.

Synthesis of 12 from 13—A mixture of 13 (1.5 g), P_4S_{10} (2.5 g), pyridine (35 ml), and H_2O (0.2 ml) was heated under reflux for 4 h. The solution was evaporated to dryness and hot H_2O (60 ml) added. The mixture was boiled for 15 min, and the crystals that separated on cooling were filtered off and chromatographed on silica gel (80 g) using $CHCl_3$ -MeOH (20:1) as an eluent. Fractions containing the triacetate of 12 were concentrated and the residue was treated with Et_2O to give the triacetate of 12 as an amorphous powder (640 mg, 41%), mp 128–132°C. This product (0.4 g) was dissolved in 20% NH_3 -MeOH (10 ml) and the solution was kept at room temperature overnight and then evaporated to dryness. The residue was recrystallized from 70% EtOH to give 12 (0.2 g, 67%), mp 236–240°C (dec.), undepressed on admixture with a specimen of 12 derived from 11.

Reaction of 7 with PhNHCN in NH_3 -MeOH—A solution of 7 (100 g) and PhNHCN¹⁶⁾ (110 g) in 20% NH_3 -MeOH (1.5 l) was heated at 150°C for 5 h in an autoclave. The brown reaction mixture was evaporated to dryness and the residue was triturated with EtOH (500 ml) to give a crystalline solid. Recrystallization from hot H_2O (10 l) gave crude 1 as brown needles (34 g). Recrystallization of these from 20% EtOH and then from hot H_2O afforded pure 1 as colorless needles (31 g, 21%), mp 247–248°C. *Anal.* Calcd for $C_{16}H_{18}N_6O_4$: C, 53.62; H, 5.06; N, 23.45. Found: C, 53.45; H, 4.99; N, 23.24.

The mother liquor, after isolation of crude 1, was concentrated to a small volume and kept at 0°C for several days. Compound 15 separated slowly as stout rods (21 g), mp 239–240°C (dec.) lit.¹²⁾ mp 245–247°C from 50% EtOH. UV: λ_{max}^{PH} 251, 290 nm, λ_{max}^{PH} 255, 278 nm.

Reaction of 7 with PhNHCN in Methanolic Propylamine—In the manner described for the above experiment, 7 (1 g), PhNHCN (1.2 g), and 33% *n*-Pr NH_2 -MeOH (30 ml) were heated and the colored solution was evaporated to dryness to give a brown resin. It was triturated with $CHCl_3$ and the deposited solid was filtered off. Recrystallization from hot H_2O afforded 1 (0.3 g) as pale yellow needles, mp 245–248°C.

Reaction of 7 with N,N' -Diphenylcarbodiimide (17)—A mixture of 7 (1 g), 17 (2 ml), and 20% methanolic ammonia (20 ml) was heated at 180°C for 5 h in an autoclave. The reaction mixture was evaporated to dryness and the residue was crystallized from EtOH to give 1 (150 mg), mp 243–245°C.

Reaction of 7 with Triphenylmelamine (19)—Compound 19 was obtained from PhNHCN according to Arndt's method.¹⁴⁾ In a manner similar to that described for the experiment using PhNHCN, a mixture of 7 (1 g), 19 (mp 224–225°, 1.2 g), and 20% NH_3 -MeOH (20 ml) was treated to give 1 (0.25 g), mp 243–245°C.

Ethyl 5-Amino-1-(β -D-ribofuranosyl)imidazole-4-carboximidate (21b)—Epichlorohydrin (4.95 g) was added dropwise to a stirred and ice-cooled solution of $BF_3 \cdot OEt_2$ (47%, 15 g) in anhydrous Et_2O (40 ml). The solution was left at room temperature for 30 min, whereupon a viscous resin ($Et_3O^+ \cdot BF_4^-$) separated. The supernatant liquid was decanted, and the resin was washed with Et_2O , and dissolved in CH_2Cl_2 (40 ml). A solution of 4 (10 g) in CH_2Cl_2 (60 ml) was added dropwise to this solution under ice-cooling and stirring. After cooling overnight, the reaction mixture was diluted with $CHCl_3$ (300 ml) and washed with cold aq. $NaHCO_3$ twice. The organic layer was concentrated to give the tri-O-propionate of 21b as a viscous oil (10.6 g, 100%).

The oil (10 g) was dissolved in 20% NH_3 -MeOH (150 ml) and kept at 0°C overnight. The solution was concentrated to 20 ml and cooled. It deposited crystals of 21b (3.2 g, 67%), which were recrystallized from MeOH- Et_2O to yield colorless crystals, mp 179–181°C. *Anal.* Calcd for $C_{11}H_{18}N_4O_5$: C, 46.15; H, 6.34; N, 19.59. Found: C, 45.68; H, 6.43; N, 19.01. MS m/e : 286 (M^+). NMR (DMSO- d_6) δ : 1.29 (3H, t, CH_3CH_2), 4.25 (2H, q, CH_3CH_2), 5.44 (1H, d, H_1'), 7.38 (1H, s, H_2).

Reaction of 21b with PhNHCN—A mixture of 21b (1 g), PhNHCN (1.2 g), and 20% NH_3 -MeOH (30 ml) was heated at 150°C for 5 h. The reaction mixture was evaporated to dryness and the residue was chromatographed on silica gel (30 g) using $CHCl_3$ -MeOH (10:1) as an eluent. The fractions containing 1 were collected and the solvent was evaporated off to give crystals of 1 (0.27 g), mp 244–246°C.

Acknowledgement The authors are grateful to Drs. K. Morita and Y. Sanno for encouragement throughout this work. Thanks are also due to the members of the analytical section for analyses and spectral measurements.

References and Notes

- 1) R.M. Berne, *Am. J. Physiol.*, **204**, 317 (1963).
- 2) H. Van Belle, *FEBS Letters*, **3**, 133 (1969).
- 3) L.B. Cobbin, R. Einstein, and M.H. Maguire, *Brit. J. Pharmacol.*, **50**, 25 (1974); E. Schaumann and W. Kutscha, *Arzneim.-Forsch.*, **22**, 783 (1972); J.A. Angus, L.B. Cobbin, R. Einstein, and M.H. Maguire, *Brit. J. Pharmacol.*, **41**, 592 (1971); W. Jahn, *Arzneim.-Forsch.*, **19**, 701 (1969).
- 4) W. Jahn, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **251**, 95 (1969); R.N. Prasad, A. Fung, K. Tietje, H.H. Stein, and H. Brondyk, *J. Med. Chem.*, **19**, 1180 (1976); R.N. Prasad, D.S. Bariana, A. Fung, M. Savic, and K. Tietje, *J. Med. Chem.*, **23**, 313 (1980).
- 5) R. Marumoto, Y. Yoshioka, O. Miyashita, S. Shima, K. Imai, K. Kawazoe, and M. Honjo, *Chem. Pharm. Bull.*, **23**, 759 (1975).

- 6) K. Kawazoe, N. Matsumoto, M. Tanabe, S. Fujiwara, M. Yanagimoto, M. Hirata, and K. Kikuchi, *Arzneim.-Forsch.*, **30**, 1083 (1980).
- 7) K. Imai, R. Marumoto, K. Kobayashi, Y. Yoshioka, J. Toda, and M. Honjo, *Chem. Pharm. Bull.*, **19**, 576 (1971).
- 8) K. Suzuki and I. Kumashiro, Japan Patent 5225 (1969) [C.A., **71**, 12561n (1969)].
- 9) E.C. Taylor and A. McKillop, "The Chemistry of Cyclic Enaminonitriles and *o*-Aminonitriles," John Wiley and Sons, Inc., New York-London, 1970, p. 306.
- 10) E. Muller, "Neuere Anschauungen der organischen Chemie," Zweite Auflage, Springer Verlag, Berlin-Gottingen-Heidelberg, 1957, p. 442.
- 11) "Beilsteins Handbuch der organischen Chemie," Vierte Auflage, Vol. 12, Verlag von Julius Springer, Berlin, 1929, p. 368.
- 12) J. Davoll and B.A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).
- 13) In fact, reaction of **7** with phenylguanidine gave **1** in good yield. The details of the reaction will be presented elsewhere by Y. Sawa *et al.*
- 14) F. Arndt, *Ann. Chem.*, **384**, 350 (1911).
- 15) P.C. Srivastava, G.A. Ivanovics, R.J. Rousseau, and R.K. Robins, *J. Org. Chem.*, **40**, 2920 (1975).
- 16) A.W. Hofmann, *Ber.*, **18**, 3217 (1885).